brum than in the cerebellum in normal mice ($p < 0.001$). The D-serine level in the cerebellum was significantly higher in the mutant than in normal mice ($p < 0.01$). The D/L ratio in the cerebrum was high in both the normal and the mutant mice. The high D-serine content in the cerebrum of the normal mice is probably due to the low DAAO activity. The D/L ratio in the cerebellum, was much lower in the normal mice as compared to the mutant animals. As to other amino acids, only the D-enantiomer of alanine was shown to be present. The D/L ratios were 0.0170 and 0.0348, respectively, in the cerebrum and cerebellum of the mutant animal, and 0.0055 and 0.0030, respectively, in the control mouse. Taken together, it appears that DAAO is mostly involved in the catabolism of D-serine.

DAAO is localized in Bergmann glial cells and astrocytes[11]: the greater proportion of the activity is in the glial spaces around the various kinds of synapses. D-serine is known to modulate N-methyl-D-aspartate (NMDA) receptor-mediated responses. D-Serine applied iontophoretically to rat thalamus neurons may have a dual action, a facilitation of NMDA receptor-mediated responses and a non-selective inhibitory action[12]. The effect of a depressant reagent on root potentials of rat spinal cord was reversed by D-serine in vitro[13]. Hence, it seems possible that DAAO plays a role in the mouse nervous system. The physiological meaning of the presence of large amounts of D-serine in the cerebrum is a matter for further study.

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Methimazole treatment reduces cardiac hypertrophy and mortality without a concomitant reduction in blood pressure in established Goldblatt two-kidney one clip hypertension

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Abstract. The effects of methimazole, an antithyroid drug, on blood pressure and other parameters were evaluated in the established phase of Goldblatt two-kidney one clip (G2K-1C) hypertension. Methimazole was administered via drinking water for five weeks, starting five weeks after hypertension had been induced. After this period of treatment, similarly high blood pressures were observed in methimazole-treated and non-treated G2K-1C rats, despite the fact that a hypothyroid state had been achieved in methimazole-treated rats. Methimazole-treated G2K-1C rats showed reductions in heart rate, ventricular weight, ventricular/body weight ratio and mortality in comparison with rats not treated with methimazole. These results clearly demonstrate that hypothyroidism induced by methimazole: a) does not reverse G2K-1C hypertension, but b) improves the rate of survival and c) reduces relative cardiac hypertrophy, possibly by the reduction in cardiac work observed in Goldblatt hypothyroid rats.
Key words. Goldblatt two-kidney one clip hypertension; methimazole; hypothyroidism; relative cardiac hypertrophy.

Thyroidectomy and antithyroid drugs reduce high blood pressure in experimental hypertension produced in several different ways, despite differences in the underlying pathophysiological mechanisms. This reduction is observed especially in the early phase of hypertension. Thus, when a hypothyroid state is produced in young spontaneously hypertensive rats (SHR), or simultaneously with the induction of hypertension by experimental means, it is able to prevent genetic[2,3], DOCA-salt[4,5], low-renal-mass[6], renal[7], and Goldblatt two-kidney one clip (G2K-1C)[5] hypertension. However, in the established phase of hypertension, discrepancies have been observed depending on the model[2,8], and the duration[3] of hypertension.

We found methimazole to be effective both in preventing and in reversing low-renal-mass hypertension[6] (low renin model).

The present study was primarily designed to confirm that methimazole would also reverse G2K-1C hypertension, which is initiated by hyperactivity of the renin-angiotensin system secondary to renal ischemia (renin-dependent model). However, the data demonstrated that while methimazole markedly reduced thyroid hormone levels, it did not reverse G2K-1C hypertension as it had done when we used a similar protocol in the low-renal-mass model[6].

Materials and methods

G2K-1C hypertension was induced in male Wistar rats initially weighing 100–125 g by placing a 0.2-mm silver clip on the left renal artery under ether anesthesia. The right kidney was left untouched. Rats subjected to a sham operation served as controls (C, n = 9).

Five weeks after the induction of hypertension, hypertensive rats were divided into two groups; G2K-1C methimazole-treated (GM, n = 9) and untreated (G, n = 11) rats. At this time, systolic blood pressure measured by plethysmography in both groups was 178 ± 9 mm Hg and 176 ± 9 mm Hg, respectively. Methimazole was administered at a dose of 0.025 mg/100 ml via drinking water. Five weeks after methimazole treatment, the femoral artery was cannulated for direct blood pressure and heart rate recordings, and to extract blood samples. Systolic blood pressure was measured weekly by tail plethysmography in unanesthetized rats. At least five determinations were made for each rat during each recording session, and the mean of the lowest three pressures within 5 mm Hg was used to calculate the systolic blood pressure value. Direct mean arterial pressure (MAP) and heart rate (HR) were measured at the end of the experiment through the femoral artery catheter in conscious rats 24 h after implantation of the catheter (TRA-021 transducer connected to a two-channel Letigraph 2000 recorder, Letica SA Barcelona, Spain). After allowing 30 min for stabilization, we used the recordings from the last five minutes to calculate MAP and HR. Subsequently, blood samples were taken and the kidney and heart were weighed. Plasma parameters measured were: T_4, T_3, sodium, potassium, urea and creatinine levels.

Plasma T_4 and T_3 levels were determined by ELISA (immunoassay system, Baxter, Miami, USA). Sodium, potassium, urea and creatinine were measured on the day of sampling by an autoanalyzer (Beckman CX4, USA). Comparisons of each parameter were carried out with one-way ANOVA analyses. When the overall ANOVA was significant, we then performed pairwise comparisons with Bonferroni's and Newmann-Keul's method. The correlation coefficient was computed to test the association between relative cardiac hypertrophy and estimated heart work.

Results and discussion

Figure 1 shows the final MAP and HR (direct recording) in control, G and GM rats. Increased MAP and HR was observed in G rats with respect to controls. Blood pressure in GM rats was similar to that in untreated G rats, and HR was markedly decreased. The blood pressure data agreed with the weekly values obtained previously by tail plethysmography (data not shown). These results demonstrate that methimazole did not reverse G2K-1C hypertension. However, a mortality rate of 30% was observed in G rats, whereas none of the methimazole-treated rats died.

The effectiveness of methimazole treatment in decreasing thyroid activity was confirmed by the reduced T_4 and T_3 plasma levels in GM rats. An increased plasma potassium in G animals and a decreased plasma creatinine in the GM group were also seen. G rats showed an increase in ventricular weight and ventricular/body weight ratio (VW/BW) when compared with control rats. A significant reduction in these parameters was observed in GM rats when compared with untreated G rats, indicating that the degree of relative cardiac hypertrophy was reduced by the antithyroid drug (table).

To evaluate the relationship between hypertension and methimazole treatment on the development of cardiac hypertrophy, VW/BW ratios were plotted as a function of cardiac work estimated as the product of HR × MAP[9]. The three groups were pooled to produce a common regression line. The data identified a correlation between VW/BW ratio and estimated heart work ($r = 0.74$; $p < 0.001$) (fig. 2). This suggests that ventricular weight varied as a function of cardiac work rather than as a function of blood pressure. Moreover, these data indicated that treatment with methimazole in the established phase of this type of hypertension reduced cardiac hypertrophy and heart work to a similar degree.

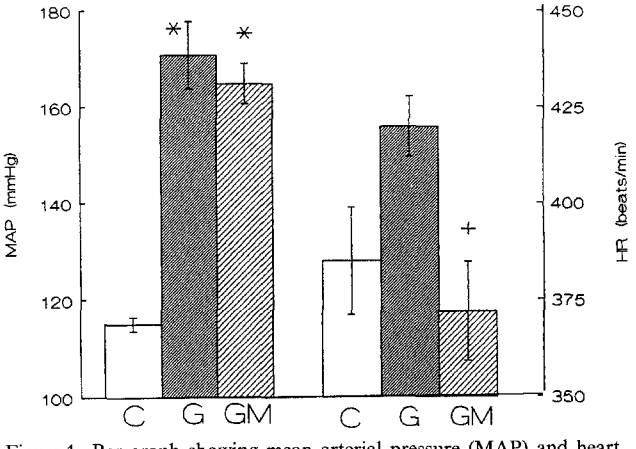

Figure 1. Bar graph showing mean arterial pressure (MAP) and heart rate (HR) measured by direct recording in conscious control (C), Goldblatt two-kidney one clip (G) and Goldblatt two-kidney one clip methimazole-treated (GM) rats. * $p < 0.001$ compared with controls; + $p < 0.01$ compared with Goldblatt untreated rats.

Body weight (BW), ventricular weight (VW), ventricular/body weight ratio (V/B), plasma sodium, potassium, urea, creatinine (CR), thyroxine and triiodothyronine in control (C), Goldblatt two-kidney one clip (G) and Goldblatt two-kidney one clip methimazole-treated (GM) rats.

Groups	BW (g)	VW (mg)	V/B (mg/g)	Na^+ (mEq/l)	K^+ (mEq/l)	Urea (mg/dl)	Cr (mg/dl)	T_4 (mg/dl)	T_3 (ng/dl)
C (n = 9)	376 ± 8.6	897 ±28	2.38 ±0.05	146 ±0.7	4.5 ±0.1	30 ±1.2	0.46 ±0.02	3.7 ±0.17	45 ± 7.2
G (n = 8)	336 ±14.3	1203** ±49	3.63** ±0.25	144 ±1.2	5.6 ±0.2	43 * ±4.7	0.50 ±0.02	4.3 ±0.17	53 ± 8.3
GM (n = 9)	344 ±14.8	910 $^+$ ±16	2.66 $^+$ ±0.07	146 ±0.85	4.0 $^+$ ±0.15	*37 ±1.5	*0.32 $^+$ ±0.02	**0.0 $^{++}$	**00 $^{++}$

Data are mean ± SEM, * $p < 0.01$, ** $p < 0.001$ compared with control group; $^+$ $p < 0.01$, $^{++}$ $p < 0.001$ compared with Goldblatt group.

In a previous report[5] we observed that the induction of a hypothyroid state, by ^{131}I administration simultaneously with the implantation of the silver clip, prevented the development of G2K-1C hypertension. However, the results of the present work demonstrated that hypothyroidism induced by methimazole did not significantly reduce blood pressure in established G2K-1C hypertension. These results contrast with previous observations in low-renal-mass hypertension, where the hypothyroidism induced by methimazole was shown to be equally effective in decreasing blood pressure in both the early and established phases of hypertension[6].

All studies reported to date concur that hypothyroidism is able to prevent the development of different types of experimental hypertension in rats[2-7]. However, in the established phase of genetic[2] and DOCA-salt[4] hypertension thyroidectomy failed to normalize blood pressure. Nevertheless, although blood pressure remained at hypertensive levels, a marked reduction in the mortality rate was observed in hypertensive hypothyroid rats[4]. This phenomenon was confirmed in our methimazole-treated group.

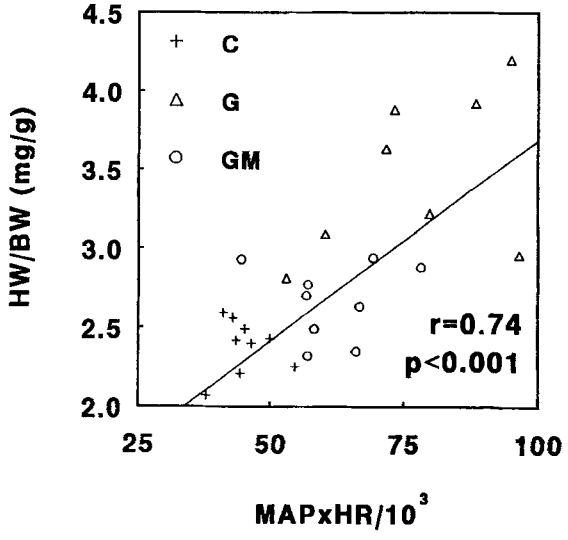

Figure 2. Relationship between ventricular/body weight ratio (VW/BW) and estimated heart work + controls; ▵ Goldblatt two-kidney one clip hypertensive; ○ Goldblatt methimazole-treated rats. Each point corresponds to an individual animal at the time of death.

The reasons for the varying effects of hypothyroidism in the established phase of hypertension of different types has not been investigated. Morphological changes as well as modifications in the synthesis of collagen or its distribution in the vascular wall may be involved[10]. It is also possible that methimazole may produce its hypotensive effect in low-renal-mass hypertension through a toxic effect absent in Goldblatt hypertension: since the drug was administered via drinking water in both cases, in low-renal-mass rats this may have resulted in an overdose due to their polydipsia-polyuria syndrome. However, the fact that another antithyroid drug administered via rat chow reversed hypertension in rats[9] speaks against this possibility.

An important cardiovascular manifestation of hypothyroidism is a reduction in HR. Reduced HR can contribute to the hypotensive effect of hypothyroidism, decreasing cardiac output. However, GM rats showed a marked reduction in HR, but their blood pressure was not modified. These results indicate that the reduction in HR is not in itself responsible for the hypotensive effect of hypothyroidism in experimental hypertension. In this regard, Rioux and Berkowith[2] also observed that HR may be severely reduced without restoring normotension in 10-week-old thyroidectomized SHR.

Arterial hypertension is commonly associated with relative cardiac hypertrophy. However, a marked reduction in VW as well as in the VW/BW ratio were noted in GM rats, so that cardiac hypertrophy was dissociated from hypertension. This dissociation has also been observed in DOCA-salt[4] hypertension, where hypothyroidism reverses cardiac hypertrophy regardless of the effect on blood pressure.

In summary, the results of this study demonstrate that treatment with methimazole: a) does not reverse G2K-1C hypertension, b) reduces relative cardiac hypertrophy and c) improves the survival rate in chronic Goldblatt hypertension. Hence, thyroid hormones are not required for the expression of this type of hypertension in its established phase.

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Phosphoramidon enhances allatostatin-mediated inhibition of juvenile hormone biosynthesis in the corpora allata of the cockroach, *Diploptera punctata*

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Abstract. Use of the enkephalinase inhibitor phosphoramidon in the in vitro radiochemical assay for juvenile hormone biosynthesis enhanced allatostatin-mediated inhibition of hormone production by corpora allata of the cockroach, *Diploptera punctata*. Significant increases in inhibition in day 2 virgin female CA by AST 1 (at 10^{-7}M) and AST 4 (10^{-8}-10^{-7}M) were observed in the presence of phosphoramidon (10^{-5}M or greater). No significant increases in inhibition were seen in CA from day 6 mated females with AST 4 (10^{-9}-10^{-7}M) and phosphoramidon combined. Phosphoramidon alone had no effect on JH biosynthesis. Analysis of allatostatin content of the CA, as determined by ELISA, revealed that addition of phosphoramidon to the medium increased the endogenous allatostatin content in CA of virgin and mated females. The similarity in primary structure between allatostatins and enkephalin-like peptides and their similar distribution makes it probable that phosphoramidon acts by preventing breakdown of allatostatins within the CA.
Key words. Corpora allata; juvenile hormone biosynthesis; allatostatins; phosphoramidon; enkephalinase; cockroach; *Diploptera punctata*; ELISA.

Allatostatins (ASTs), isolated from brains of the cockroach *Diploptera punctata*, are potent inhibitors of juvenile hormone (JH) biosynthesis by the corpora allata (CA) in vitro[2-5]. These peptides are probably important in the regulation of JH production by the CA and as such, may be involved in the regulation of reproduction in the adult female cockroach. AST 1 has been the most extensively studied with regard to its physiological effects. Sensitivity of CA to AST 1 varies during the first reproductive cycle of the adult female, but the greatest inhibition of in vitro rates of JH biosynthesis by AST 1 occurs in CA from day 2 virgin and day 6 mated females[3,5]. However, the inhibitory effect is neither stage- nor species-specific, and both larval CA and those of mated *Periplaneta americana* females are also inhibited to some degree[2,3].

Allatostatins are neuropeptides of up to 18 amino acids[2,4] and have amino acid sequences that show some similarity to the vertebrate enkephalin-related peptide Met-enkephalin-Arg[6]-Gly[7]-Leu[8] (met-8). Brains, CA and corpora cardiaca (CC) of *D. punctata* contain cells which show immunoreactivity to met-8 antiserum; these cells may contain allatostatin-like substances with cross-reactivity to the antibody[6]. Met-8 (**Tyr-Gly**-Gly-**Phe**-Met-Arg-**Gly-Leu**) has 4 or 5 amino acids in similar sequence, with deletions, to the C-terminus of allatostatin 1 (-Tyr-Gly-Phe-Gly-Leu-NH_2). Therefore, substances influencing the metabolism of met-8 or the enkephalins may be expected to exert similar effects on the metabolism of the allatostatins.

Enkephalins and related peptides are subject to enzymatic degradation by enkephalinases. Sequence similarity between the allatostatins and the enkephalins raises the possibility that they are also cleaved by enkephalinase-like enzymes that may be present in the brain, CA or CC. N-[α-L-rhamnopyranosyloxyhydroxyphosphinyl]-L-leucyl-L-tryptophan (phosphoramidon) is a specific inhibitor of enkephalinase (E.C. 3.4.24.11, endopeptidase-24.11) enzymatic activity[7]. Since the primary structures of the allatostatins and enkephalins are similar, phosphoramidon may also protect the allatostatins from degradation (table 1). It would therefore have an enhancing effect on allatostatin-mediated inhibition of juvenile hormone biosynthesis in the cockroach CA. Our experiments were designed to test this possibility and to determine if phosphoramidon could be used to improve the effectiveness of our assay for inhibition of JH biosynthesis.

In this regard, we have tested the effect of either substitutions in the sequence or truncations in the length of the